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EXAMINER

DUNSTON, JENNIFER ANN

ART UNIT	PAPER NUMBER
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1636

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/692,553	Applicant(s) COURT ET AL.	
	Examiner Jennifer Dunston	Art Unit 1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 07 March 2008.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1,3-10,12,13 and 22-26 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1,3-10,12,13 and 22-26 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 23 August 2006 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

This action is in response to the amendment, filed 3/7/2008, in which claim 11 was canceled, and claims 12 and 24 were amended. Currently, claims 1, 3-10, 12-13 and 22-26 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected of Group I without traverse in the reply filed on 12/5/2005. Currently, claims 1, 3-10, 12-13 and 22-26 are under consideration.

Priority

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosures of the prior-filed applications, International Application No. PCT/US01/25507 and Provisional Application Nos. 60/225,164 and 60/271,632, fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The prior-filed application numbers do not provide literal or inherent support for the claimed method steps of claims 1-13 and 22. While the prior-filed applications suggest that the disclosed method of homologous recombination may be used to construct complex conditional targeting vectors, the specifications do not set forth the claimed method steps. For example, the prior-filed applications do not provide adequate written description for the method step of using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of second recombining sites and a first recombining site into a second site into the gene in a bacterial artificial chromosome. The prior-filed applications do not teach how to use the disclosed recombination system to make a vector for the conditional knockout of a gene, where two first recombining sites remain in a gene and recombination of the two first sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

Claims 1-10, 12-13 and 22-26 have an effective filing date of 2/12/2003.

Response to Arguments - Claim Objections

The objection of claims 12 has been withdrawn in view of Applicant's amendment to the claim in the reply filed 3/7/2008.

Response to Amendment - Declaration of "Dr. Pentao Liu"

The declaration under 37 CFR 1.132 filed 3/7/2008 is insufficient to overcome the rejection of claims 24-26 based upon the Casanova reference as set forth in the last Office action.

At paragraph 3, the declaration notes that a declaration under 37 C.F.R. § 1.131 is of record. Paragraph 3 of the present declaration notes that the prior declaration describes the introduction of a second pair of recombining sites flanking a second selectable marker into a vector that already includes a first pair of recombining sites. This results in the presence of one first recombining site and two second recombining sites. It is noted that claim 1 requires the introduction of a first pair of recombining sites flanking a selectable marker and requires excising the nucleic acid marker such that a single first recombining site remains in the gene. Thus, the introduction of the second selectable marker flanked by a second pair of recombining sites results in the presence of one first recombining site and two second recombining sites. This is not the invention claimed in claims 24-26. These claims further limit the method of claim 1 to require two first recombining sites and two second recombining sites. The additional first recombining site of the claims is introduced at the same time as the second pair of recombining sites. This additional first recombining site is not shown by either declaration. As noted in paragraph 3 of the present declaration, the prior declaration documents the use of a first and second pair of recombining sites. However, claims 24-26 require a first pair of recombining sites, and a second pair of recombining sites further comprising an additional first recombining site (five sites total as compared to the four sites shown by the declaration).

The additional data referred to in paragraph 4 of the present declaration fails to show the additional first recombining site required by claims 24-26.

The declarations of record do not provide sufficient evidence to demonstrate an actual reduction to practice of the claimed invention prior to the date of the Casanova et al reference

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 24-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Casanova et al (Genesis, Vol. 32, No. 2, pages 158-160, Published Online 2/13/2002, cited in a prior action; see the entire reference) in view of Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 9/10/2007 and is reiterated below.

Casanova et al teach a method for generating a vector for conditional knockout of a gene, comprising the following steps: (i) co-electroporating a BAC construct and a kanamycin cassette flanked by two LoxP sites (LoxP-Kan-LoxP) into *E. coli* JC8679 competent cells, (ii) selecting for kanamycin resistant clones, (iii) transforming the BAC DNA, from a bacterial colony that had undergone homologous recombination, into Cre-expressing bacteria to excise the nucleic acid encoding the selectable marker, which leaves a single LoxP site in the gene (iv) co-electroporating into *E. coli* JC8679 competent cells the BAC DNA comprising the single LoxP site and a plasmid comprising a FRT-PGK_{Th5}neo-FRT-loxP flanked by two homology arms, and (v) transforming the resulting recombinant BAC into FLP-expressing bacteria to excise the

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marker gene (e.g. page 158, left column, 2nd full paragraph; page 158, paragraph bridging columns; Figure 1). Casanova et al teach the use of ET-cloning (homologous recombination in *E. coli* to insert the nucleic acid molecules encoding a selectable marker into the BAC construct (e.g. Figure 1). Casanova et al teach that the recombination of the remaining two first recombining sites will produce a nucleic acid sequence that cannot be transcribed to produce a functional protein (e.g. page 158, left column, 2nd paragraph). Casanova et al teach the abovementioned method, where the first recombining sites comprise a LoxP site, and the second recombining sites comprise a FRT site. Casanova et al teach the use of markers that confer resistance of the cell to an antibiotic such as kanamycin (e.g. Figure 1). Further, Casanova et al teach the use of Cre-expressing and FLP-expressing bacteria as taught by Buchholz et al (1996).

Casanova et al do not teach homologous recombination wherein the cell comprises the pL promoter operably linked to a nucleic acid encoding Beta, Exo and Gam and wherein the first recombination site comprise a FRT site.

Lee et al teach a PL operon encoding beta, exo and gam under the control of the temperature-sensitive λ repressor (allele cI857) for use in BAC engineering (e.g. page 56, right column, 1st full paragraph; page 57, left column, 1st full paragraph). Further, Lee et al teach the use of the recombination system in combination with the flpe gene under the control of the P_{BAD} inducible promoter (e.g. strain EL250; Table 1). Lee et al teach that the recombination system is highly efficient and can produce recombination frequencies that are at least 50- to 100-fold higher than those obtained with plasmid based systems (e.g. page 64, left column, last paragraph). Lee et al teach the recombination of an FRT-kan-FRT cassette into the mouse *Eno2* gene within a BAC vector (e.g. page 57, *Construction of plasmids*; Figure 1). Lee et al teach that

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the use of flpe provides a higher recombination frequency than the original flp gene (e.g. page 60, left column, 1st paragraph). With regard to the use of the recombination system for the construction of conditional targeting vectors, Lee et al state the following:

This recombination system also facilitates the generation of complicated conditional targeting vectors. While the generation of such vectors often used to take several months, it can now be performed in only a few weeks. The ability to express reversibly Cre or Flpe recombinases in *E. coli* speeds this process even further. A selectable marker flanked with *loxP* or *FRT* sites can now be introduced into an intron of a gene and then be removed by transient Cre or Flpe expression, leaving behind a solo *loxP* or *FRT* site in the intron. See page 64, right column, 2nd paragraph.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Casanova et al to include the phage lambda recombination system taught by Lee et al because Casanova et al and Lee et al teach it is within the ordinary skill in the art to use homologous recombination in *E. coli* to engineer BAC vectors to produce conditional targeting constructs. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method to use FRT sites as the first recombination site and LoxP as the second recombination site because Casanova et al teach it is within the ordinary skill in the art to use of a LoxP-Kan-LoxP cassette to insert a single LoxP site and Lee et al teach it is within the ordinary skill of the art to use a FRT-Kan-FRT cassette to insert an FRT site into the intron of a gene. Further, Lee et al teach that the LoxP and FRT sites can be used interchangeably.

One would have been motivated to make such a modification in order to receive the expected benefit of increased efficiency of homologous recombination and FRT site-specific recombination, which would decrease the amount of time required to make the targeting construct, as taught by Lee et al. Further, one would have been motivated to use FRT in place of

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LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 24-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Casanova et al (Genesis, Vol. 32, No. 2, pages 158-160, Published Online 2/13/2002, cited in a prior action; see the entire reference) in view of Stewart et al (US Patent No. 6,355,412, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 9/10/2007 and is reiterated below.

The teachings of Casanova et al are described above and applied as before.

Casanova et al do not teach homologous recombination wherein the cell comprises the pL promoter operably linked to a nucleic acid encoding Beta, Exo and Gam and wherein the first recombination sites comprise a FRT site.

Stewart et al teach a method of performing homologous recombination in a host cell, comprising introducing a nucleic acid sequence encoding RecE/T or Red α/β recombinase (i.e. Lambda Exo and Beta) into a host cell, introducing a polynucleotide comprising a nucleotide sequence homologous to the nucleotide sequence of interest into the host cell, activating the expression of RecE/T, and selecting a cell from the population in which homologous recombination has occurred (e.g. column 28, lines 10-50; column 29, lines 9-35; column 28, line 51 to column 29, line 8; columns 25-27; paragraph bridging columns 37-38). Further, Stewart et

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al teach the use of Gam in addition to Exo and Beta or RecE/T (e.g. column 25, lines 5-28; Example 1). Stewart et al teach that a variety of host-vector systems may be utilized to introduce and express the protein-coding sequence of RecE/T or Red α/β , including prokaryotic and eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect or mammalian cells (e.g. column 28, lines 10-40). With respect to regulatory controls, Stewart et al teach that a range of different expression levels and a variety of regulatory sequences are known in the art and the ability to generate a wide range of expression is advantageous for utilizing the method (e.g. column 25, lines 5-44; column 24, line 50 to column 25, line 3). Stewart et al teach that the expression can be regulated by the P_L promoter of phage λ and the inducible lambda repressor CI₈₅₇ (e.g. column 26, lines 1-27). Stewart et al teach that the nucleotide sequence of interest may be extrachromosomal and located on a bacterial artificial chromosome (e.g. column 20, lines 37-57; paragraph bridging columns 28-29). Moreover, Stewart et al teach that the lambda recombinases can be used to achieve high-efficiency targeted cloning (e.g. column 11, lines 3-47).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Casanova et al to include the lambda beta, exo and gam genes operably linked to the pL promoter as taught by Stewart et al because Casanova et al and Stewart et al teach it is within the ordinary skill in the art to use homologous recombination to modify BAC constructs in a cell. Further, it would have been obvious to use a FRT site as the first recombining site, because Casanova et al teach the use of both LoxP and FRT sites for the same purpose.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to conduct high efficiency recombination in a variety of host cell types as taught by Stewart et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 3-10, 12, 13, 22 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al (J. Clin. Invest. Vol. 98, No. 3, pages 600-603, August 1996, cited in a prior action; see the entire reference) in view of Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 9/10/2007 and is reiterated below.

Rajewsky et al teach a method for generating a vector for conditional knockout of a gene in a cell, comprising the steps of (i) using homologous recombination to insert a nucleic acid construct encoding a first loxP site and a selectable marker flanked with loxP sites, (ii) excising the selectable marker with Cre, a recombinase specific for the loxP recombining sites flanking the selectable marker, wherein recombination of the remaining recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein, thereby generating the vector for conditional knockout of the gene in the cell (e.g., paragraph bridging pages 6001-602; page 602, left column, 1st full paragraph; Figure 1A).

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Rajewsky et al do not teach two separate steps for the introduction of the three loxP sites in that Rajewsky et al do not teach using homologous recombination to insert a nucleic acid encoding a first selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome, excising the selectable marker with a first recombinase, and using homologous recombination to insert a nucleic acid encoding a second selectable marker flanked by a pair of second recombining sites. Further, Rajewsky et al do not teach the cell comprising a de-repressible promoter operably linked to a nucleic acid encoding Beta, Exo and Gam, wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta, Exo and Gam.

Lee et al teach a PL operon encoding beta, exo and gam under the control of the temperature-sensitive λ repressor (allele cI857) for use in BAC engineering (e.g. page 56, right column, 1st full paragraph; page 57, left column, 1st full paragraph). Further, Lee et al teach the use of the recombination system in combination with the flpe gene under the control of the P_{BAD} inducible promoter (e.g. strain EL250; Table 1). Lee et al teach that the recombination system is highly efficient and can produce recombination frequencies that are at least 50- to 100-fold higher than those obtained with plasmid based systems (e.g. page 64, left column, last paragraph). Lee et al teach the recombination of an FRT-kan-FRT cassette into the mouse *Eno2* gene within a BAC vector (e.g. page 57, *Construction of plasmids*; Figure 1). Lee et al teach that the use of flpe provides a higher recombination frequency than the original flp gene (e.g. page 60, left column, 1st paragraph). With regard to the use of the recombination system for the construction of conditional targeting vectors, Lee et al state the following:

This recombination system also facilitates the generation of complicated conditional targeting vectors. While the generation of such vectors often used to

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take several months, it can now be performed in only a few weeks. The ability to express reversibly Cre or Flpe recombinases in *E. coli* speeds this process even further. A selectable marker flanked with *loxP* or *FRT* sites can now be introduced into an intron of a gene and then be removed by transient Cre or Flpe expression, leaving behind a solo *loxP* or *FRT* site in the intron. See page 64, right column, 2nd paragraph.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Rajewsky et al to include the phage lambda recombination system and bacterial artificial chromosome (BAC) modification taught by Lee et al, because Rajewsky et al and Lee et al teach it is within the ordinary skill in the art to use homologous recombination in to produce conditional targeting constructs and Lee et al teach that BACs can be used as targeting substrates. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the single step of homologous recombination of Rajewsky et al to be two separate steps, where the first *loxP* site is inserted by a separate homologous recombination event followed by site-specific recombination to remove the marker, and where the second pair of *loxP* sites flanking the selectable marker are introduced by a homologous recombination event, because the application of the method of Lee et al would result in the same structure as taught by Rajewsky et al, and Lee et al specifically teach that the system can be used to make conditional knockout vectors. Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method to use *FRT* sites in place of *loxP* as the second recombination site because Lee et al teach it is within the ordinary skill of the art to use a *FRT*-Kan-*FRT* cassette to insert an *FRT* site into the intron of a gene. Further, Lee et al teach that the *LoxP* and *FRT* sites can be used interchangeably.

One would have been motivated to make such a modification in order to receive the expected benefit of increased efficiency of homologous recombination and FRT site-specific recombination, which would decrease the amount of time required to make the targeting construct, as taught by Lee et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 3-10, 12-13, 22 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al (J. Clin. Invest. Vol. 98, No. 3, pages 600-603, August 1996; see the entire reference) in view of Muyrers et al (TRENDS in Biochemical Sciences, Vol. 26, No. 5, May 2001, cited on the IDS filed 10/23/2003; see the entire reference) and Stewart et al (US Patent No. 6,355,412, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 9/10/2007 and is reiterated below.

The teachings of Rajewsky et al are described above and applied as before.

Rajewsky et al do not teach two separate steps for the introduction of the three loxP sites in that Rajewsky et al do not teach using homologous recombination to insert a nucleic acid encoding a first selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome, excising the selectable marker with a first recombinase, and using homologous recombination to insert a nucleic acid encoding a second

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selectable marker flanked by a pair of second recombining sites. Further, Rajewsky et al do not teach the cell comprising a de-repressible promoter operably linked to a nucleic acid encoding Beta, Exo and Gam, wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta, Exo and Gam.

Muyrers et al suggest the modification of bacterial artificial chromosomes to generate mouse knockout constructs (e.g., page 325, right column). Further, Muyrers et al teach that recombinogenic engineering often involves two rounds of processing, where the first round uses homologous recombination to generate an initial product by integration of a selectable gene, together with additional functional elements at the intended site, and the second round makes use of the extra functional elements to remove the selectable gene, thereby generating the final product (e.g., page 325, right column). Muyrers et al teach that the application of this process can be used to accomplish virtually any DNA alteration (e.g., page 325, right column). Muyrers et al specifically teach the use of site-specific recombination sites that are recombination sites for Cre or FLP recombinases to flank selectable cassettes to leave a single site-specific recombination target site at a particular location (e.g., page 326, paragraph bridging columns; Figure 1(c)). Moreover, Muyrers et al teach that this is a highly efficient way to eliminate the selectable cassette and it is also a useful method of placing a site-specific recombination site exactly where it is required (e.g., page 326, right column). Muyrers et al teach that recombinogenic engineering can be carried out by lambda Beta ($\text{Red}\beta$), lambda Exo ($\text{Red}\alpha$) and lambda Gam ($\text{Red}\gamma$) (e.g., pages 328-329, Recombinogenic engineering using ET recombination). Muyrers et al teach that it is desirable to limit the recombinogenic window by

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regulating the expression of one or more of the components of the recombination system (e.g., pages 329-330, Limiting the recombinogenic window).

Stewart et al teach a method of performing homologous recombination in a host cell, comprising introducing a nucleic acid sequence encoding RecE/T or Red α/β recombinase (i.e. Lambda Exo and Beta) into a host cell, introducing a polynucleotide comprising a nucleotide sequence homologous to the nucleotide sequence of interest into the host cell, activating the expression of RecE/T, and selecting a cell from the population in which homologous recombination has occurred (e.g. column 28, lines 10-50; column 29, lines 9-35; column 28, line 51 to column 29, line 8; columns 25-27; paragraph bridging columns 37-38). Further, Stewart et al teach the use of Gam in addition to Exo and Beta or RecE/T (e.g. column 25, lines 5-28; Example 1). Stewart et al teach that a variety of host-vector systems may be utilized to introduce and express the protein-coding sequence of RecE/T or Red α/β , including prokaryotic and eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect or mammalian cells (e.g. column 28, lines 10-40). With respect to regulatory controls, Stewart et al teach that a range of different expression levels and a variety of regulatory sequences are known in the art and the ability to generate a wide range of expression is advantageous for utilizing the method (e.g. column 25, lines 5-44; column 24, line 50 to column 25, line 3). Stewart et al teach that the expression can be regulated by the P_L promoter of phage λ and the inducible lambda repressor CI₈₅₇ (e.g. column 26, lines 1-27). Stewart et al teach that the nucleotide sequence of interest may be extrachromosomal and located on a bacterial artificial chromosome (e.g. column 20, lines 37-57; paragraph bridging columns 28-29). Moreover, Stewart et al teach that when the recombination method is used in combination with site-specific recombination sites, the site-

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specific recombinase, which recognizes the sites is under the control of an inducible promoter such that upon induction of recombination expression, recombination between the site-specific recombination sites occurs (e.g., Figure 4). Stewart et al teach that the lambda recombinases can be used to achieve high-efficiency targeted cloning (e.g. column 11, lines 3-47).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Rajewsky et al to include the phage lambda recombination system and bacterial artificial chromosome (BAC) modification taught by Muyrers et al, because Rajewsky et al and teach it is within the ordinary skill in the art to use homologous recombination in to produce conditional targeting constructs and Muyrers et al teach that lambda-mediated recombination can be used to modify BACs for mouse targeting constructs or to accomplish virtually any DNA alteration. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the single step of homologous recombination of Rajewsky et al to be two separate steps, where the first loxP site is inserted by a separate homologous recombination event followed by site-specific recombination to remove the marker, and where the second pair of loxP sites flanking the selectable marker are introduced by a homologous recombination event, because the application of the method of Muyrers et al would result in the same structure as taught by Rajewsky et al, and Muyrers et al specifically teach that the system can be used to make virtually any alterations. Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method to use FRT sites (recognized by Flp recombinase) or loxP sites (recognized by Cre recombinase). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the expression of the

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lambda-mediated recombination proteins such that they are under the control of the de-repressible P_L promoter of phage λ , because Muyrers et al teach that regulatable expression is desirable, and Stewart et al teach the use of the P_L promoter of phage λ to regulate the expression of the lambda recombination proteins. It would have also been obvious to perform the recombination events in a prokaryotic cell or eukaryotic cell as taught by Stewart et al, because Muyrers et al teach the use of lambda-mediated recombination, and Stewart et al teach that this method can be performed in prokaryotic or eukaryotic cells.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to conduct high efficiency recombination in a variety of host cell types as taught by Muyrers et al and Stewart et al to arrive at a conditional targeting vector with the structure taught by Rajewsky et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Arguments - 35 USC § 103

With respect to the rejection of claims 24-26 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Lee et al, Applicant's arguments filed 3/7/2008 have been fully considered but they are not persuasive.

The response essentially asserts that the Casanova et al publication is removed as a reference by the declaration under 37 C.F.R. § 1.131, as supported by the declaration of Dr. Liu under 37 C.F.R. § 1.132. This reference has not been removed for the reasons of record and the reasons set forth above with respect to the insufficiency of the declaration of Dr. Liu.

The response asserts that Lee et al do not suggest, or render obvious inserting a second nucleic acid encoding a selectable marker flanked by a pair of second recombining sites into a second site in the gene. This is not found persuasive, because this limitation is taught by Casanova et al. Thus, each of the limitations of the rejected claims is met by the combined teachings of Casanova et al and Lee et al.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

With respect to the rejection of claims 24-26 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Stewart et al, Applicant's arguments filed 3/7/2008 have been fully considered but they are not persuasive.

The response essentially asserts that the Casanova et al publication is removed as a reference by the declaration under 37 C.F.R. § 1.131, as supported by the declaration of Dr. Liu under 37 C.F.R. § 1.132. This reference has not been removed for the reasons of record and the reasons set forth above with respect to the insufficiency of the declaration of Dr. Liu.

The response essentially asserts that Stewart et al do not teach features of the claimed invention. This is not found persuasive, because Casanova is available as prior art and is relied upon for these teachings. Thus, each of the limitations of the rejected claims is met by the combined teachings of Casanova et al and Stewart et al.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

With respect to the rejection of claims 1, 3-10, 12, 13, 22 and 23 under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al in view of Lee et al, Applicant's arguments filed 3/7/2008 have been fully considered but they are not persuasive.

At pages 9-10, the response asserts that Rajewsky et al is directed to classical gene targeting and not homologous recombination to make a gene targeting vector, and, thus, there is not rationale to combine Rajewsky et al with any references that teaches recombineering in bacterial cells. At page 11, the response asserts that there is no motivation or suggestion, in either Rajewsky et al or Lee et al, to use methods for generating vectors for a conditional knock out that include the use of two selectable markers, each flanked by a pair of recombining sites. In response to applicant's argument that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, the knowledge to apply homologous recombination to the modification of a vector is taught by Lee et al, and the rationale to combine the references is provided by Lee et al. Lee et al specifically teach the introduction of a selectable marker flanked by two recombining sites and teach the use of the recombination system to generate complicated conditional targeting vectors (e.g., page 64, right column, 2nd paragraph). Rajewsky et al also teach that a selectable

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marker flanked by two recombining sites can be used in the construction of a conditional targeting vector (page 601, left column) and provide a specific structure for a more complicated conditional targeting vector (Figure 1A). One could readily envision using the method of Lee et al to arrive at the conditional targeting vector structure provided by Rajewsky et al, where the selectable marker flanked by a pair of recombining sites is inserted, the markers are combined, and a second selectable marker flanked by a second pair of recombining sites is inserted. One would have been motivated to use the method of Lee et al to decrease the amount of time it takes to make the vector as compared to classical gene targeting methods.

At page 10, the response asserts that there is no information in Rajewsky et al with regard to methods for producing the vectors shown in Figure 1, use of a BAC to produce the vector, the introduction of a first selectable marker flanked by a pair of first recombining sites into a BAC, use of two pairs of recombining sites, and excision of a first selectable marker with Cre during the generation of the targeting vector. In summary, the response asserts that the teachings of Rajewsky et al are a “far cry from the claimed methods of producing vectors.” In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Rajewsky et al teach a vector for conditional knockout of a gene in a cell, and teach the use of homologous recombination to obtain a chromosome comprising this vector (e.g., page 601).

At page 11, the response asserts that one of skill in the art would only be motivated to use homologous recombination induced by the PL promoter operably linked to Beta and Exo (as

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described by Lee et al) to produce a targeting construct including three recombining sites (as described by Rajewsky et al). This is not found persuasive, because Lee et al teach the removal of the selectable marker by recombination. The response asserts that there is a myriad of ways, each with a myriad of steps, that could be used to accomplish this goal, and there is no motivation, suggestion, or inference in either Rajewsky et al or Lee et al to suggest or render obvious the specific steps of (1) using homologous recombination to insert a second nucleic acid encoding a second selectable marker flanked by a pair of second recombining sites into a second site in the gene; followed by (2) excising the nucleic acid encoding the selectable marker with a second recombinase specific for the second recombining sites; and (3) excising the selectable marker such that the targeting vector cannot be transcribed to produce a functional protein. This is not found persuasive, because, as discussed above, Lee et al and Rajewsky et al both teach that recombination of sites flanking a selectable marker to excise the nucleic acid encoding the selectable marker. Rajewsky et al teach the structure of a conditional gene targeting vector (Figure 1A). One could readily envision the creation of the structure taught by Rajewsky et al by applying two rounds of the homologous recombination method of Lee et al with a selectable marker flanked by recombining sites. Lee et al specifically suggest the use of this method to generate complicated conditional targeting vectors (e.g., page 64, right column, 2nd paragraph). It is noted that the claimed method does not require the excision of the selectable marker such that the targeting vector cannot be transcribed to produce a functional protein. The claim can be reasonably interpreted such that the phrase "wherein recombination of the recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein" does not refer to the excising steps of the method but instead refers to the fact that the location of the

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remaining recombination sites must be capable of performing this function. The method is drawn to generating a targeting vector for conditional knockout of a gene in a cell. Thus, the vector must not already have a mutation that prevents the production of a functional protein. Conditional vectors allow functional protein to be produced until the remaining recombining sites are recombined in the transgenic animal comprising the vector. As explained by Rajewsky et al, conditional gene targeting allows one to achieve cell type-specific and/or inducible gene targeting (i.e., the mutation is not created in the germline of the mouse and thus the mutation is not directly introduced by the targeting vector) (e.g., page 601, right column, 1st full paragraph).

The response asserts that Rajewsky et al do not teach any methods for generating a vector for conditional knockout, and thus the Examiner's statement that it would be obvious to "modify the single step of homologous recombination of Rajewsky et al to be two separate steps..." is difficult to understand. The Examiner apologizes for the confusing statement. It would be obvious to use two separate steps to generate the structure of the conditional targeting vector disclosed by Rajewsky et al.

The response asserts that (1) the prior art does not include each element as claimed, as the prior art does not describe the introduction of a second selectable marker flanked by a pair of second recombining sites; (2) the prior art could not have combined the elements as claimed with known methods, since Rajewsky et al only describe homologous recombination in ES cells with a targeting vector that only includes three recombining sites, and Lee et al teach recombination methods that are functional only in bacterial ES cells; and (3) there is no evidence that the results of the combination would be predictable. This is not found persuasive. Upon reading the Rajewsky and Lee references one of skill in the art could readily envision the application of two

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rounds of targeting taught by Lee et al to create the structure taught by Rajewsky et al. Lee et al specifically teach the excision of the selectable markers subsequent to targeting. Thus, the combination of Rajewsky et al and Lee et al results in the introduction of a second selectable marker flanked by a pair of recombining sites. The assertion that Rajewsky et al only describe homologous recombination in ES cells does not influence how one would consider constructing a conditional targeting vector. As acknowledged by Applicant on pages 11-12 of the response Rajewsky et al do not specifically teach how the targeting vector was made. One would look to the teachings of Lee et al to make the vector. Lee et al specifically suggest the use of their homologous recombination method to generate complicated conditional targeting vectors, and thus there is a reasonable expectation of success. There is no evidence of record to support the assertion that this application would be unpredictable.

At page 16, the response asserts that the specification provides evidence of unexpected superior properties of the claimed methods. The response asserts that none of the prior art references disclose a vector comprising a BAC. This argument does not show unexpected results. Furthermore, this limitation is met by Lee et al. The response notes that the specification describes the method as rapid and efficient. This is not an unexpected property of the claimed method. Lee et al teach that the method of making a vector by homologous recombination is rapid and efficient (e.g., page 64, left column; last paragraph; page 64, right column, second full paragraph). The response notes that it takes less than two weeks to construct a vector using the method disclosed in the specification. Lee et al state that it takes only a few weeks, as compared to the prior art methods, which take several months. Thus, the prior art recognized this beneficial property and it is not unexpected.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

The rejection of claim 11 under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al in view of Muyrers et al and Stewart et al is moot in view of Applicant's cancellation of the claims.

With respect to the rejection of claims 1, 3-10, 12-13, 22 and 23 under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al in view of Muyrers et al and Stewart et al, Applicant's arguments filed 3/7/2008 have been fully considered but they are not persuasive.

The response indicates that Rajewsky et al is discussed on pages 8-13 of the reply with respect to the combination of Rajewsky et al and Lee et al. These arguments are not found persuasive for the reasons set forth above.

The response notes that Muyrers et al describe recombinogenic processing in two rounds comprising (1) insertion of a single unit of DNA including a selectable marker; and (2) the removal of that selectable marker. This is what is claimed in terms of inserting a selectable marker flanked by a pair of first recombining sites, and excising the selectable marker. Further, Muyrers et al state, "By informed application of these principles, virtually any DNA alteration can be accomplished." See page 325, right column, 2nd full paragraph.

The response asserts that Muyrers et al statement that the "scar" of a single recombining site "can be a problem if left in protein coding or regulatory regions" teaches away from the claimed invention. This is not found persuasive, because one of skill in the art would have the common sense not to select these regions for targeting. For example, Rajewsky et al show the placement of recombination sites upstream of regulatory regions and in introns (e.g., Figure 1).

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The selection of alternative sites other than coding sequences and regulatory regions was well within the skill of the art at the time the invention was made, and Muyrers et al do not disparage the use of these locations.

The response again asserts that the “second round” taught by Muyrers et al is very different than repeating the entire process a second time. Muyrers et al teach that recombinogenic engineering often involves two rounds of processing, where the first round uses homologous recombination to generate an initial product by integration of a selectable gene, together with additional functional elements at the intended site, and the second round makes use of the extra functional elements to remove the selectable gene, thereby generating the final product (e.g., page 325, right column). Muyrers et al teach that the application of this process can be used to accomplish virtually any DNA alteration (e.g., page 325, right column). Given the teachings of Rajewsky et al, one of skill in the art would easily envision repeating the entire two rounds of Muyrers et al twice to result in the DNA alteration disclosed by Rajewsky et al.

The response asserts that Muyrers et al teach away from the use of recombining sites and teach entirely different methods for introducing nucleic acids of interest. Thus, the response refers to the combination of Muyrers et al and Rajewsky et al as an impermissible combination. This is not found persuasive, because a reference is prior art for all that it teaches and additional disclosed embodiments do not constitute a teaching way from another disclosed embodiment. Accordingly, the combination of Muyrers et al and Rajewsky et al is not precluded by any teaching away.

The response asserts that Stewart et al do not teach using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of first recombining sites into

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a first site in a gene in a bacterial artificial chromosome, wherein a vector comprises the bacterial artificial chromosome. This is not found persuasive, because the rejection is based upon the combined teachings of Rajewsky et al, Muyrers et al and Stewart et al. This limitation is met by the teachings of Muyrers et al.

The response asserts that Stewart et al do not teach excising the nucleic acid encoding the selectable marker with a first recombinase specific for the first recombining sites, wherein a single first recombining site remains in the gene, or using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a second recombining sites into a second site in the gene, or excising the nucleic acid encoding the selectable marker with a second recombinase specific for the second recombining sites such that two first recombining sites remain in the gene following excision of the nucleic aid encoding the selectable marker, wherein recombination of the two first recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The response asserts that there is no motivation or suggestion, in either Rajewsky et al, Muyrers et al, or Stewart et al, to use methods for generating vectors for a conditional knockout that included the use of two selectable markers, each flanked by a pair of recombining sites. This is not found persuasive, because Rajewsky et al specifically teach conditional targeting vectors, and Muyrers et al teach the use of selectable markers flanked by a pair of recombining

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sites to make virtually any DNA alteration, including the generation of mouse knockout constructs. Given the recognized efficiency of the use of a selectable marker flanked by recombination sites (e.g., Muyrers et al, page 326, right column), one would have been motivated to use this method of altering DNA to make the structure disclosed by Rajewsky et al.

The response assert that the prior art does not include each element as claimed, as none of Rajewsky et al, Muyrers et al, or Stewart et al describe methods that include either the introduction of a second selectable marker or a second pair of recombining sites. Given the combined teachings of Rajewsky et al, Muyrers et al and Stewart et al, one of skill in the art at the time the invention was made would have recognized that the insertion of a selectable marker flanked by recombining sites, followed by excision of the selectable with a recombinase would be performed twice in order to place recombining sites around sequences that result in loss-of-function of a gene in order to make a conditional targeting vector as taught by Rajewsky et al.

The response asserts that the prior art could not have combined the elements as claimed by known methods, since Rajewsky et al only teach homologous recombination in ES cells with a targeting vector that includes three recombining sites, and Stewart et al and Muyrers et al teach recombination methods that are functional only in bacterial cells, and Muyrers et al teach away from using recombining sites. However, Applicant notes on page 11 of the reply that the recombination in ES cells taught by Rajewsky et al is not for the construction of a targeting vector. Thus, one would not look to this for how to make the vector. Rather, one would have looked to the teachings of Muyrers et al and Stewart et al, neither of which teaches away from the use of recombining sites (see discussion above).

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The response asserts that there is no evidence to support that the claimed methods would be predictable. This is not found persuasive, because Muyrers et al teach that the use of recombination sites flanking a selectable marker can be used to make virtually any DNA alteration (e.g., page 325, right column). Applicant has not provided any evidence to contradict the teachings of Muyrers et al.

At page 16, the response asserts that the specification provides evidence of unexpected superior properties of the claimed methods. The response asserts that none of the prior art references disclose a vector comprising a BAC. This argument does not show unexpected results. Furthermore, this limitation is met by Muyrers et al and Stewart et al. The response notes that the specification describes the method as rapid and efficient. This is not an unexpected property of the claimed method. Muyrers et al teach that the use of site specific recombinases flanking a selectable marker followed by recombination is a highly efficient method of placing a site specific recombination site exactly where it is required (e.g., page 326, right column). Thus, one would expect it to only take a couple of weeks to construct the vector for conditional knockout of a gene in a cell. This expectation is supported by Lee et al, who teach that it takes only a few weeks to make a conditional knockout vector with this method, as compared to the prior art methods, which take several months. Thus, the prior art recognized this beneficial property and it is not unexpected.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached at 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished

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applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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Examiner
Art Unit 1636

/JD/

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